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Genetic Contribution to DZ Twinning

Cathryn M. Lewis, Sue C. Healey, and Nicholas G. Martin

Department of Medical Informatics (C.M.L.), University of Utah, Salt Lake City, Utah; and Queensland Institute of Medical Research (S.C.H., N.G.M.), Brisbane, Australia

The genetic contribution to dizygotic (DZ) twinning was investigated using 6,596 twin pairs from the Australian Twin Registry who provided information on other twins in their families. Responses were classified by the zygosity (DZ; monozygotic [MZ]) of the proband twins and by the relationship and zygosity of related twins. MZ probands and MZ twins reported by DZ probands were used as controls and assumed to be independent of any genetic influence. Significantly higher proportions of DZ twins were found in the families of DZ probands compared to the families of MZ probands for the following relationships: sibs of probands, proband mothers, offspring of sisters of proband mothers, and offspring of female probands (*P* < 0.001 in each case). The latter 2 relationships were used to estimate risk ratios of 1.7 for sisters of mothers of DZ twins, and 2.5 for offspring of female DZ twins. No greater tendency to DZ twinning in close relatives was found in mothers who bore DZ twins at a younger age than at an older age.

KEY WORDS: monozygotic twins, dizygotic twins, genetics, relative risks

INTRODUCTION

The existence of a genetic contribution to dizygotic (DZ) twinning has long been known through the identification of families with a strong predisposition to twins [Weinberg, 1909], although the magnitude of this effect and the mode of inheritance of DZ twinning are still uncertain. White and Wyshak [1964] used Mormon records of the offspring of DZ twins to show that female DZ twins had a twinning rate of 17.1 per 1,000 maternities, and male twins only 7.9; they also found higher DZ twinning rates in the sisters of DZ twins compared to the brothers of DZ twins. Bulmer [1970] summarised data from several studies to estimate relative risks of 1.8 in the mothers/daughters of mothers of DZ twins, and 2.6 in their sisters, using data pooled from several studies. The existence of a paternal influence in twinning has been the subject of much controversy. Greulich [1934] and Parisi et al. [1983] both showed an excess of DZ twins in paternal relatives of DZ twins, although the first study was certainly biased by under-reporting of singleton births in paternal relatives.

The mode of inheritance of DZ twinning was assumed to be recessive by Weinberg [1909] and Wyshak and White [1965]; Bulmer [1970] used the relative risks quoted above to postulate that DZ twinning was inherited as a recessive gene with frequency 0.5 and penetrance 5% in susceptible women. A recent pedigree analysis of data from 1,422 DZ probands in the Netherlands Twin Register (NTR) and the East Flanders Prospective Twin Study (EFPTS) indicates that the inheritance of DZ twinning is compatible with a dominant model with gene frequency of 0.03 and penetrance of 10% [Meulemans et al., 1993]. In Booroola merino sheep, a codominant gene for multiple ovulation has been mapped to the ovine homologue of human chromosome 4q21-25 [Montgomery et al., 1993]. The strongest influences on DZ twinning are maternal age, parity, and race, but little is known of the relative importance of, or the interactions between, genetic factors, and these pregnancy- or population-related factors.

In contrast, monozygotic (MZ) twinning is generally accepted to be a spontaneous event, uninfluenced by genetic factors, maternal age, parity, or race. The NTR/EFPTS pedigree data showed no increase in MZ twinning in kindreds that were ascertained through MZ proband twins, supporting the opinion that MZ twinning is a sporadic trait [Meulemans, 1994]. However, Segreti et al. [1978] report several pedigrees where MZ twinning appears to be inherited and in their larger and more systematic italian study, Parisi et al. [1983] found support for a modest genetic influence on MZ twinning. The report by Derom et al. [1987] of an increased frequency of MZ twins following artificial ovulation induction suggests a mechanism by which the 2 types of twinning may be related and a possible explanation for any modest genetic influence on MZ twinning.

This analysis uses data from twins in the Australian Twin Registry who were asked to give details of the zygosity and exact relationship of other sets of twins in

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Address reprint requests to Cathryn M. Lewis, Ph.D., Genetic Epidemiology, 391 Chipeta Way, Suite D2, Salt Lake City, UT 84108.

their family. No limitation was given on the distance of relationships to be included or the number of pairs of twins that could be reported. The aim of this analysis is to detect and quantify the genetic contribution to DZ twinning. This will be performed through comparison of the numbers of pairs of twins reported in different relationships, stratified by the proband and related twins' zygosity. The data collected here are selected through original entry into the registry and through response to the questionnaire or telephone interview. Since singleton birth data were only collected for the sibship containing the proband twins and for the offspring of the proband twins, rates of DZ or MZ twinning in relatives cannot be calculated for most relationships of interest. But comparison of occurrences of DZ and MZ related twins for DZ/MZ probands is valid, under certain assumptions of internal consistency that will be discussed below. For all the analyses that follow, MZ twinning will be treated as a sporadic event, so that MZ twins can be regarded as random population controls for predisposition to DZ twinning, and MZ relatives of DZ probands should occur at random.

MATERIAL

The Australian Twin Registry (ATR) was established in 1978 and consists of over 20,000 pairs of DZ and MZ self-reported twins who have participated in a variety of studies. In 2 large mailed questionnaire studies covering many aspects of health and lifestyle, adult (>18 years) twins were asked "Are there any other twins in your family? If YES, please state exact relationship and whether twins are identical or non-identical." The first study, of twins born 1893–1964 (cohort 1), was conducted in 1980–81 and complete replies were received from 3,808 pairs (64%) of the 5,967 pairs mailed. The twins were followed up in 1992–93 in a telephone interview focused on psychiatric symptoms, the final section of which concerned confirmation of family twinning history. The second study, of twin pairs born 1963–1971 (cohort 2), was conducted in 1989 and was answered by one or both twins of 2,788 pairs (85%) from the 3,269 who were contactable. No systematic telephone follow-up was conducted, but probands reporting twin cousins without specifying the exact relationship (e.g., mother's sister's offspring) were phoned for clarification. Data are available from either 1) interview with both twins, 2) interview with one twin only, or 3) written questionnaire only. Details of the sample size and the numbers of respondents in each category are given in Table I. The twins registered in the ATR will be referred to as "proband twins" and their relatives as "related twins."

TABLE I. Respondent Data From Cohort 1 and Cohort 2

	Cohort 1	Cohort 2
Number of responses		
DZ	2,009	1,654
MZ	1,799	1,134
Type of response		
Interview (2 twins)	2,549	0
Interview (1 twin)	625	0
Questionnaire only	634	2,788
Date of birth		
Mean	1947	1967
Range	1893–1964	1963–1971

The zygosity of the proband twins in the ATR was determined through a similarity questionnaire which has previously been found to have over 95% specificity and sensitivity [Martin and Martin, 1975; Kasriel and Eaves, 1976]. The zygosity of the related twins was accepted as that reported by the proband twins, and an unknown zygosity was recorded for inconsistent reporting from proband co-twins. Where telephone interviews were conducted, the usual questions on physical similarity and being mistaken for each other were asked about each related same-sex twin pair. Adopted proband twins were omitted from the analysis. Summary response data were stratified by zygosity and sex (DZF, DZM, DZO, MZF, MZM) of the proband pair (Table II). Fewer male twins admitted to having twin relatives than female twins (55% compared to 70%). However, for those male twins who did report related twin pairs, the mean number of pairs reported was similar to that reported by the female twins (3.00 compared to 2.97).

Data were collected from proband twins themselves, although for a study of genetic influences on DZ twinning, we are concerned with the relationships between parents of the proband and related twins, with particular reference to the mothers of DZ twins. Relationships were coded so that each type of first degree relative of the proband parents could be identified. For example, first cousin twins were defined as being through the mother's sister, the mother's brother, the father's sister,

TABLE II. Response Data From Pooled Cohorts, by Zygosity

Zygosity[a]	Number of proband twin pairs	% with related twins	Mean number of related twin pairs[b]	% with related twins of zygosity[b]	
				DZ	MZ
DZF	1,274	70	3.14	62	31
DZM	774	53	3.15	64	27
DZO	1,615	73	3.09	65	30
MZF	1,900	70	2.85	50	38
MZM	1,033	57	2.90	47	40
	6,596				

[a]DZF: female dizygotic twins; DZM: male dizygotic twins; DZO: opposite sex dizygotic twins; MZF: female monozygotic twins; MZM: male monozygotic twins.
[b]Including only probands with related twins. Some related twins of unknown zygosity were reported.

the father's brother or through unknown intervening relatives (relationships 13–19, Table III). Similarly, for twin nieces/nephews of the proband twins it is important to distinguish whether the parent was a brother or sister of the proband twins (relationships 5–7, Table III). More distant relationships were categorised only as being a maternal or paternal ancestor of the proband twins (relationships 23–24). Table III lists the classifications, together with the total number of proband twins who reported a related twin in that category, regardless of the zygosity information given. Figure 1 illustrates the major categories of related twin parents and their relationship to the proband twins.

The data will be analysed from the perspective of the proband twin, and whether the proband twin has, or does not have, a related twin of specified zygosity and relationship. Data were initially stratified by the cohort and collection method (0, 1, or 2 telephone interviews). It was clear that data from the written questionnaires contained more incomplete relationships (for example, "cousin") and more unknown zygosities. When the frequency of twins of various summary categories (for example, parents or cousins) were compared across the cohort and collection method, no differences were detected. This was confirmed through recontacting the 156 cohort 2 twins who had originally reported a first cousin, without completely specifying the relationship (i.e., categories 15, 18, 19). Of the 133 pairs who were contacted, 106 confirmed the cousin relationship; and of these, 39, 24, 20, and 23 were reclassified into the categories 13, 14, 16, and 17, respectively. One cousin could only be classified as relationship 15. Of the other 26 pairs, 22 had no twin first cousins, only more distant twins, and 4 had aunts/uncles who were twins. It appeared that "cousin" was distributed among the four categories for mother's sister, mother's brother, father's sister, or father's brother (13, 14, 16, and 17) in the same proportions as probands reporting this relationship exactly.

The numbers of probands who reported MZ or DZ related twins are shown in Table II, stratified by the proband zygosity. There is a clear non-independence of proband and related twins' zygosities ($P < 0.001$). This will be due in part to the genetic influence on DZ twinning, so that DZ probands are more likely to have a DZ relative. However, there is also a difference in MZ relatives, with a higher proportion of MZ probands than DZ probands reporting MZ relatives. This may indicate either a genetic susceptibility or a reporting bias, where MZ twins are more likely to denote distant relatives as MZ, and DZ twins are more likely to denote distant relatives as DZ. These data are pooled over all the relationships from Table III. Specific reporting differences in close relationships are given in Table IV and discussed below.

METHODS

Frequency tables of the numbers of MZ and DZ probands that reported MZ and DZ related twins in selected categories of Table III were constructed. Any re-

TABLE III. Classification of Relationships, and Number of Probands Who Reported a Related Twin in Each Class

Class	No. of pairs	Type of relationship[a]
1	249	Offspring of female twin
2	118	Offspring of male twin
3	0	Offspring of twin, unknown sex
4	472	Sibs of proband twins
5	193	Offspring of twins' sister
6	114	Offspring of twins' brother
7	2	Offspring of twins' sib, unknown sex
8	149	Mother is twin
9	122	Father is twin
10	376	Mother's sib: maternal aunt/uncle
11	257	Father's sib: paternal aunt/uncle
12	61	Parent's sib, unknown maternal or paternal side
13	324	Offspring of mother's sister
14	219	Offspring of mother's brother
15	31	Offspring of mother's sib, unknown sex
16	204	Offspring of father's sister
17	197	Offspring of father's brother
18	35	Offspring of father's sib, unknown sex
19	176	First cousin—unknown maternal/paternal side
23	1946	More distant maternal relationship
24	1132	More distant paternal relationship
25	350	Unspecified relationship
26	57	Female twin's daughter had twins
27	19	Female twin's son had twins
28	21	Male twin's daughter had twins
29	14	Male twin's son had twins

[a]Other relationships including half-sibs, cousins through unknown parents, etc., were also categorised but had too few data points to analyse.

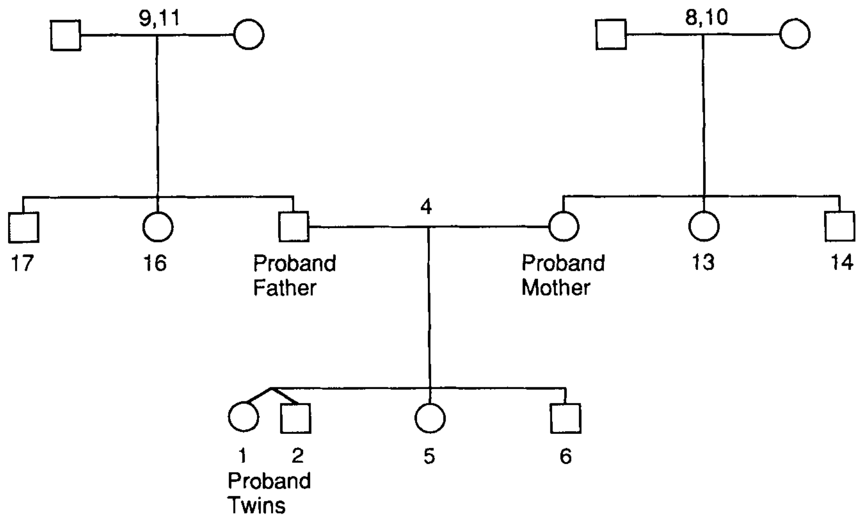

Fig. 1. Pedigree drawing showing the classification used for family members having twin offspring.

lationship with under 5 observations for specific zygosity of proband and related twins was omitted and for each relationship, the related twins of unknown zygosity have been omitted from further analysis. To investigate more closely the distribution of reported twins by proband and related twins' zygosity, a chi-square test was performed. A non-significant result from this test ($P < 0.05$, uncorrected for multiple testing), implies homogeneity of the related twin zygosities, and these relationships were not analysed further. For those relationships with a significant result, 2 binomial tests for difference in the proportions of DZ and MZ probands who reported 1) DZ twins, and 2) MZ twins were performed. For these tests, the population sample was taken to be the number of probands who responded to the questionnaire (3,663 DZ probands, 2,933 MZ probands, Table II). For categories 1 and 2 of offspring from the proband twins, the zygosity figures from Table II were used to calculate numbers of male and female probands, not pairs of twins.

Particular relationships were extracted for further analysis to investigate the existence and magnitude of a genetic contribution to DZ twinning. Twin parents or offspring and their zygosity can be assumed to be known accurately. Similarly, the twin cousins of categories 13–19 can be assumed to be known with a reasonable degree of accuracy. The parental twins will give a parent/offspring relationship, and the cousins a sibling relationship between the twin mothers. Summary data from these relationships were analysed, firstly using frequency tables and secondly using a linear model to account for different effects of the zygosities of proband and related twins and potential interactions between them.

For each relationship (cousins, parents, offspring), a linear model was fitted assuming a Gaussian distribu-

TABLE IV. Counts of Related Twins by Proband/Relative Zygosity

	No. of pairs by proband/relative zygosity				*P*-value from		
Class[a]	DZ/DZ	DZ/MZ	MZ/DZ	MZ/MZ	χ^2	P(DZ relatives)[b]	P(MZ relatives)[c]
1.	104	27	39	31	0.0008	<0.0001	0.34
2.	33	20	19	15	0.71		
4.	243	37	44	39	<0.0001	<0.0001	0.27
5.	73	25	44	15	0.86		
6.	26	19	20	20	0.62		
8.	85	9	25	21	<0.0001	<0.0001	0.008
9.	41	19	29	14	0.91		
10.	108	37	66	41	0.04	0.09	0.18
11.	56	24	49	32	0.27		
13.	123	44	53	36	0.030	0.0001	0.92
14.	76	29	54	25	0.67		
16.	69	21	46	26	0.11		
17.	59	29	49	25	0.96		
23.	466	226	320	296	<0.0001	0.03	<0.0001
24.	224	109	198	137	0.035	0.32	0.0004

[a]Relationship classes as in Table III.
[b]Testing for difference in the proportion of DZ and MZ probands that have related DZ twins.
[c]Testing for difference in the proportion of DZ and MZ probands that have related MZ twins.

tion for the number of twins (Y_{ijk}) reported by probands of zygosity i for related twins of zygosity j, in relationship class k, so that

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_{ijk} + \epsilon_{ijk}$$

where a mean number of twins μ is moderated by factors α_i for DZ/MZ proband zygosity, β_j for DZ/MZ related twins zygosity, $(\alpha\beta)_{ij}$ for a potential interaction between the zygosities of proband twins and reported twins, γ_k for a reporting bias, δ_{ijk} for a genetic influence (constrained to be zero for most i, j, k combinations), and an error term ϵ_{ijk}. The factor for proband zygosity accounted for different number of DZ and MZ probands in the original sample; the factor for related twins zygosity allowed for differing population frequencies of DZ and MZ twins. Reporting biases depended on the relationship to be analysed. For twin cousins, 2 forms of the factor was used. The first assumed that paternal relationships might be less well reported, the second allowed for different levels of bias in each cousin relationship. A similar factor for "mother" or "father" was included in the parental analysis, and "female" or "male" for the offspring analysis. The genetic factor was constrained to be zero except for relationships where a genetic influence on multiple ovulation would manifest itself (for example, DZ probands reporting a DZ mother). Risk ratios for DZ twinning were calculated for the sister of a DZ twin mother, using the raw reported data, and the genetic component from the linear models. Risk ratios for a parent/offspring relationship were calculated from DZ offspring of the male and female proband twins (categories 1 and 2); only cohort 1 probands were used, since cohort 2 probands may not have completed their families.

For cohort 1, dates of birth of the mothers and the date of birth of proband twins were available for 1,171 mothers of DZ probands. These were used to investigate whether mothers who had DZ twins at an early age were a more genetically predisposed subgroup of DZ mothers, shown by an increased frequency of DZ twins reported in relationship categories that could be exposed to the same genetic predisposition. Chi-square tests were used to test for a difference in distribution of maternal age at birth of twins and logistic regression was used to test for a decreasing probability of related DZ twins with increasing maternal age at birth.

The analysis of the proband twin data cannot be used to determine a mode of inheritance of DZ twinning, but the concordance of MZ twins for twins in their offspring can indicate the strength of the genetic component. For a highly genetic trait, both or neither MZ twins should have DZ twins. Detailed reproductive histories (singletons, twins, miscarriages) were obtained for both members of female MZ pairs in which at least one twin had reported twin offspring. Similar reproductive history was available for MZ twins in the Finnish Twin Registry (J. Kaprio, personal communication).

All analyses were performed using SAS (Cary, NC) and Splus (AT&T Bell Laboratories, Warren, NJ).

RESULTS

The chi-square analysis of related twins is shown in Table IV. Heterogeneity in the DZ:MZ ratio of related twins according to the zygosity of the proband twins was found in the following relationship categories: 1) offspring of the female twin, 4) sibs of twins, 8) mother, 10) mother's sib, 13) offspring of mother's sister, 23) more distant maternal relative, 24) more distant paternal relative. Each of these heterogeneities was highly significant ($P < 0.001$), except for the mother's sib and the more distant paternal relative ($P = 0.04$, $P = 0.035$, respectively; all P-values uncorrected for multiple testing). In these relationships, a difference in the proportion of MZ and DZ probands that reported DZ twins was found for the categories of 1) offspring of the female twin, 4) sibs of twins, and 13) offspring of mother's sister. In each case, more DZ than MZ probands reported DZ twins and no difference was found in the proportion of MZ and DZ probands who reported MZ twins. For twin mothers of proband twins, a significantly higher probability of having a DZ mother was found in DZ probands compared to MZ probands, and a significantly higher probability of having an MZ mother was found in MZ probands. The categories of more distant maternal/paternal relatives also showed a higher probability of reporting MZ twins from MZ probands than DZ probands.

A significant chi-square statistic in Table IV indicates independence of the variables in the table. This could reflect an excess in one particular cell (for example an excess of DZ probands reporting related DZ twins would suggest a genetic influence), or an interaction between the zygosity of the proband and the related twin (for example, where DZ probands were more likely to identify related twins as being DZ, and MZ probands report related twins as being MZ). In the categories where only one of the binomial proportion tests is significant, this suggests an excess of one cell in the table. Where both tests show a significant result, there may be an interaction between the zygosities of the proband and related twins. This is shown most clearly where the proband mother is a twin. The 3 categories of close relationships with an excess of DZ twinning from DZ probands are those that we would expect to be genetically influenced: offspring of the female twin, sibs of twins, and offspring of the mother's sister. The other categories that should show an excess under this test are the offspring of proband's sister and the proband mother's sibs. One concern of data quality was that cohort 2 response, from written questionnaires only, might be biased in some consistent fashion with respect to zygosities or relationships. These data have more unknown zygosities and incompletely specified relationships, but analysis in the Methods section showed that this was unbiased. Another concern is the difference in birth cohorts, so that fewer cohort 2 twins had offspring and provided relatively few observations for relationships 1–5. The analysis of Table IV was repeated using only data from cohort 1 for these relationships, and no substantially different results were found.

The risk ratios for DZ twinning for sisters are given in Tables V and VI. For the raw data (Table V), estimates were obtained from the proportion of DZ and MZ probands reporting DZ cousins in class 13 (line 1), and from comparison of numbers of different types of twin

TABLE V. Risk Ratio Estimates for Sisters of Mothers of DZ Twins (Class 13) Using Raw Reported Data

	Relatives compared			
Probands	"Cases"	"Controls"	Risk ratio	95% C.I.
1. DZ, MZ	13DZ	Non-13DZ	1.86	(1.34, 2.58)
2. DZ	13DZ	14DZ	1.62	–
3. DZ, MZ	13DZ	14, 16, 17DZ	1.70	–

cousins reported by probands (lines 2–3). The linear models (Table VI) provide estimated fits for the expected number of twin cousins reported in each of the 16 categories shown in Figure 2. As defined by Akaike's Information Criterion, the best fitting model (line 1, fitted values shown in Fig. 2) used a factor for paternal relationships to allow for fewer cousins reported in this category, and an interaction term between the proband zygosity and the reported cousin zygosity. This confirms the results of the analysis of the data in Table II that DZ probands reported more DZ relationships and MZ cousins reported more MZ relationships. DZ cousins through the mother's sister was the only category affected by this term, so the fitted value in this category is equal to the observed value (Fig. 2), although the parameters for the number of cousins reported by proband and cousin zygosities, and by cousin relationship have been estimated from the other 15 data points. This model has a significant genetic contribution ($P < 0.001$) and estimated that 49.4 of the 123 reported DZ cousins were due to the genetic contribution, which corresponds to a sister risk ratio for DZ twinning of $123/(123 - 49.4) = 1.67$. The models using a reporting factor for each cousin relationship (lines 3, 4) did not produce a significantly better fit than the models using only a paternal/maternal factor for cousin relationships. The risk ratios show a close range of values between 1.56 and 1.86.

The linear model of the twin parent data produced conflicting results. Table IV shows that the category of "mother" has an interaction, probands of each zygosity being more likely to have a mother of the same zygosity, while this effect is not present in fathers. The best fitting model assumes equal numbers of mothers and fathers of each zygosity, with a genetic effect increasing the number of DZ mothers of DZ twins (Fig. 3). This implies that the estimate of the genetic component is equal to the difference in the number of DZ mothers and the number of DZ fathers of DZ probands and so is based on only two data points. In the cousin data, reported numbers by proband zygosity, cousin zygosity, and cousin relationship were estimated from all 15 data points available, and the genetic component was then calculated from the difference between the actual and estimated value for the genetically influenced category. For the parental data, the structure of the parental reporting is more complex; the linear model suggests that the parental data should not be used for any estimation. However, parent/offspring risk ratios for DZ twinning were obtained from relationship categories 1 and 2—offspring of the male and female twins in cohort 1. The fitted numbers of DZ and MZ offspring are shown in Figure 3, under a linear model with factors for proband zygosity, offspring zygosity, proband sex and a genetic component, giving a risk ratio of 2.49, with approximate 95% C.I. (1.76, 4.29). Risk ratios were also calculated using data of twin offspring from DZ male and female proband twins (using the method of line 1, Table V). Probands who had twins of unknown zygosity were included as being non-DZ parents, and this will provide a conservative estimate of the risk ratio. For female DZ twins, a risk ratio was 2.73 (approximate 95% C.I. [1.88, 3.96]). For male DZ probands with DZ offspring, a risk ratio was 1.22 with approximate 95% confidence interval (0.69, 2.17), suggesting that sampling biases are not excessive.

Maternal age at birth of DZ twins was available for the mothers of 1,171 DZ twins. Of these, 63 reported cousins through the mother's sister, 34 mothers were DZ twins themselves, and 211 had DZ twin relatives in a genetically susceptible category (1, 4, 8, 10, and 13). However, the distribution of maternal age at birth for these mothers was not different from that of mothers who had no related twins in these categories. Furthermore, logistic regression analyses for each of these categories gave a non-significant term for maternal age.

A further estimate of the importance of genetic influence can be determined from the twin births to MZ proband twins. If there is a strong genetic influence on DZ twinning, we would expect to see high concordance rates, with both or neither MZ twins giving birth to DZ twins. In the ATR, 91 female MZ pairs were identified where at least one twin had a twin live birth, miscarriage or still birth (Table VII). Of the 33 MZ twins with DZ offspring, no co-twins had confirmed DZ twins, although one co-twin had twins of unknown zygosity, and one had a pair of MZ twins. The remaining 22 parous

TABLE VI. Risk Ratio Estimates for Sisters of Mothers of DZ Twins (Class 13) From Linear Modelling

Model components					
Reporting bias	Zygosity interaction[a]	Risk ratio	95% C.I.	Residual deviance	d.f.
1. Paternal cousins	Yes	1.67	(1.42, 2.03)	296.0	10
2. Paternal cousins	No	1.80	(1.47, 2.34)	534.7	11
3. All cousins	Yes	1.56	(1.22, 1.89)	218.4	8
4. All cousins	No	1.77	(1.36, 2.31)	490.5	9

[a]Interaction between zygosities of proband and related twins.

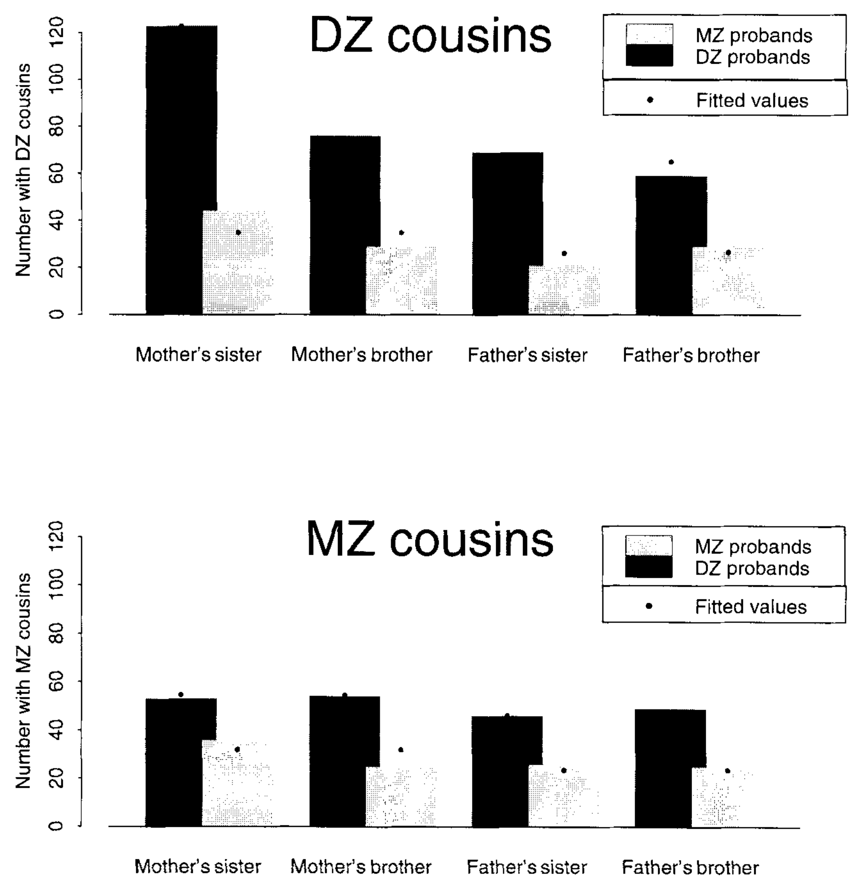

Fig. 2. Number of related twin cousins, classified by proband and related twin zygosities.

co-twins had only singleton offspring. In the Finnish Twin Registry, 18 MZ twins had opposite sex, and therefore DZ, twins. One co-twin had male twins of unspecified zygosity. Pooling the Australian and Finnish data gives two unknown zygosity twins from 41 MZ co-twins, so a maximum concordance of 2 pairs. A binomial distribution was used to calculate the maximum penetrance by mother of the DZ twinning trait such that 0, 1, or 2 DZ pairs from 41 genetically predisposed women would occur with a probability of 0.025 or less. An upper bound of 17% penetrance was obtained. This is a gross upper bound since if neither of these unknown zygosity twins were DZ, the upper bound for penetrance would be 9%. One concordant pair would give an upper bound of 13%. These figures are compatible with the 10% penetrance for a dominant model found by Meulemans et al. [1993]. It has also been suggested that DZ twinning may be associated with infertility [Allen, 1981]. The only support in these data comes from the five married but childless co-twins of mothers of DZ twins, while no childless co-twins are seen for co-twins of mothers of MZ twins, although these figures are too small for inference. Data of the number of miscarriages from the MZ mothers of twins and their co-twins were available. No difference in the number of reported miscarriages was seen between the mothers of DZ twins and mothers of MZ twins, or in their co-twins.

DISCUSSION

Previous studies to determine and quantify a genetic contribution to DZ twinning have employed a different design than this, using data from singletons and twins to calculate relative risks or twinning rates in relatives of DZ twins. Each study has had its own sources of error and bias. Weinberg [1909] dealt with small populations; Wyshak and White [1965] had adequate sample size from the Utah Population Database, but lacked zygosity data; Greulich [1934] and Parisi et al. [1983] had problems with under-reporting of singleton births. This study has taken a different approach through using a large twin registry, concentrating only on twin births and making no attempt to collect complete family structure. It can be assumed that recall of twins is more accurate than recall of singletons, especially from twins themselves. The ATR is a large registry with reasonably accurate information on the zygosity of proband twins (less than 5% error in either direction; Healey et al., in preparation]. It is difficult to estimate possible errors in zygosity determination of the related twins, but twins are probably better judges of zygosity than singletons.

The disadvantages of using this data set arise from it being a self-selected group of twins. It is possible that the probability of a twin pair belonging to the registry increases with the existence of other closely related twin pairs. Similarly, MZ twins are more interested

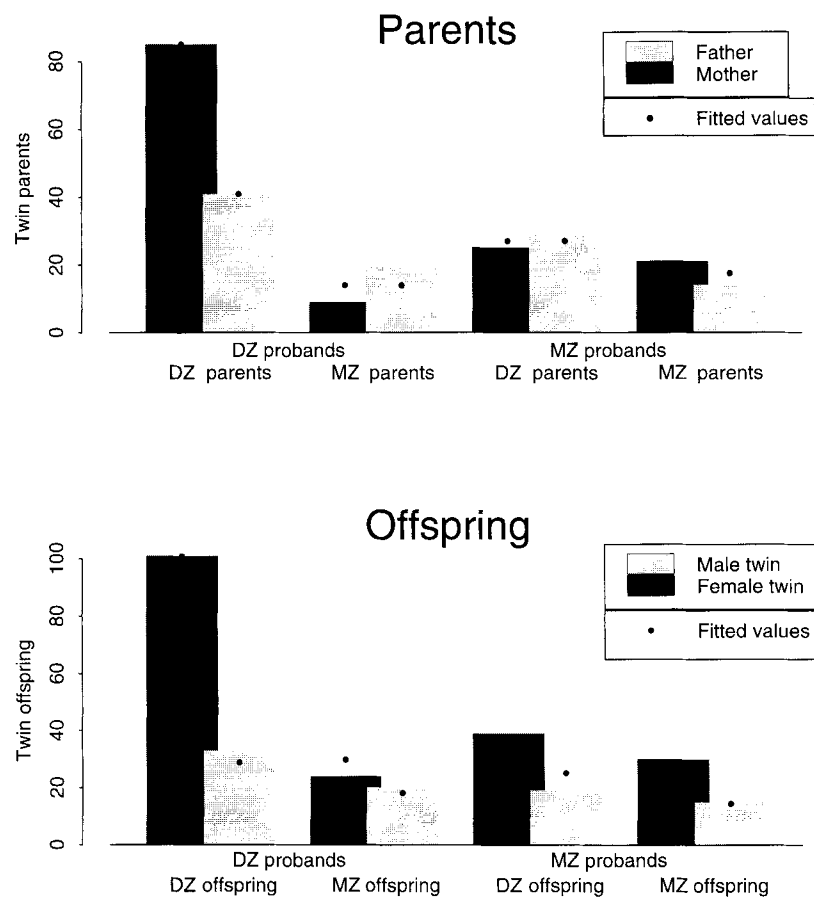

Fig. 3. Number of twin parents of MZ and DZ probands and twin offspring of MZ and DZ probands, showing fitted values from the linear models.

than DZ twins in their "twinness" and this is reflected in the relatively high numbers of MZ twins in this data set (3,663 DZ twins, 2,933 MZ twins) while population frequencies are closer to 2/3 DZ in caucasians [Bulmer, 1970]. There is some overlap between the proband twins, for example, where twin pairs are sibs, both pairs may be members of the ATR. No attempt has been made to determine relationships between probands twins, or account for this in the analysis, though we suspect the bias is slight. We will assume that the inherent nonrandomness in the data applies to both MZ and DZ probands. Response to this particular questionnaire and subsequent telephone interview should not be biased by family history of twinning, since in each case this was a single question among many others.

Only the first-degree relationships of the proband twins parents were used to classify reported twins, as it was assumed that most twins would know of twins in these relationships, and have an accurate indication of their zygosity. Some proband twins reported detailed further relationships, which have all been categorised as more distant maternal or paternal relatives. The detailed analyses used only related twins who had some indication of zygosity given. No internal check of the accuracy of the zygosity could be made, although gross inaccuracies or inconsistencies, or interactions between the zygosities of the proband and related twins, would have been seen in the analyses reported in Table IV.

It is clear from the cousin relationships in Tables III and IV that fewer twins are reported through the father's sibs than the mother's sibs. Three possible explanations of this phenomenon are i) proband twins

TABLE VII. Concordance of Twin Births From MZF Probands

	Australia MZF had—			Finland[b] MZF had—		
	DZ	MZ	XZ[a]	DZ	MZ	XZ[a]
Co-twin had—						
DZ	0	0	0	0	—	0
MZ	1	1	0	—	—	—
XZ[a]	1	0	0	1	—	0
Singletons	22	30	13	17	—	27
None						
Married	5	0	1			
Single	2	2	0			
Died young	2	1	0			
Total	33	34	14	18	—	27

[a]XZ = unknown zygosity.
[b]No zygosity information was available for the offspring of MZ twins, so twins can only be classified as opposite sex (DZ) or same sex (XZ).

may be less knowledgeable about their paternal relatives than maternal relatives. Greulich [1934] and Parisi et al. [1983] both found under-reporting of singleton births in paternal relatives, but here we are concerned with only twin births; ii) on a population level, women tend to marry earlier, and bear children at an earlier age than men. At any point in childbearing years, more twins should be seen in the father's sister's offspring than in the mother's brother's offspring. This factor will be most relevant in cohort 2, where proband twins were born 1963–1971, so sibships of the proband twin generation may not yet be completed; iii) the increase in DZ twinning at higher parities and maternal age [Bulmer, 1970] implies that maternal age at birth of MZ twins is younger than maternal age of DZ twins, so there may be a difference in the size of sibships in cousins between zygosity. The lack of difference in the number of reported cousins in the categories from mother's brother, father's sister, and father's brother suggests that this is not a significant bias, and again, would mainly be relevant for data from cohort 2. When only data from cohort 1 were analysed, similar results to those from the combined analysis were obtained.

One model for DZ twinning is that it reflects "superfertility," through high rates of multiple ovulation. Early follicular phase gonadotrophin levels (responsible for ovarian follicle recruitment, development and ovum release) are higher in mothers of DZ twins than controls [Martin et al., 1991a] and Martin et al. [1991b] found increased rates of ovulation in mothers of DZ twins compared to mothers of MZ twins who acted as controls. Mothers with a predisposition to DZ twinning may conceive more easily and more frequently, regardless of whether the conception results in a singleton or a multiple birth. So, even if the multiple ovulation does not result in a DZ twin birth, it may result in higher singleton birth rates. This would give higher fertility, not only in the mothers of DZ twins, but in their female relatives who have inherited the same genetic predisposition to multiple ovulation. In this data set, larger sibships in the relatives of DZ probands would imply more DZ and MZ twins reported, through a higher number of births and regardless of any genetic influence. This does not appear to be a substantial bias in this data set, since there are no significant differences in the proportion of DZ and MZ probands reporting MZ twins in close relationships.

DZ twinning rates increase with maternal age so it might be supposed that mothers who have their twins at a younger age are expressing a stronger genetic predisposition. However, our data show no greater familial clustering in mothers who gave birth to their DZ twins at a younger age. This implies that older onset cases are just as useful for genetic studies (segregation and linkage analysis) and that potential confounding factors of increased maternal age and parity may in fact be genetic covariates of the DZ twinning trait.

The ability of this data set to detect a genetic influence on DZ twinning is clear from Table IV, where the 6 categories found showing non-independence in the number of DZ and MZ probands reporting DZ and MZ twins in each relationship included 5 categories that would be expected to show a genetic influence given that the primary effect is on multiple ovulation. Of the 4 close relationships that were significantly heterogeneous (offspring of female twin, sibs of twins, mother, offspring of mother's sister), all showed a higher proportion of DZ than MZ probands reporting DZ twins ($P < 0.001$, in each case). Where the mother was a twin, a significantly higher proportion of MZ than DZ probands reported MZ twins. This is the only close relationship to show an MZ-influence, and may be due to reporting bias, although it is just possible that it reflects a weak genetic influence on MZ twinning reported by some authors [Harvey et al., 1977; Segreti et al., 1978; Shapiro et al., 1978]. The relationships of more distant maternal/paternal relatives both showed a strong MZ-influence, and only a marginal DZ-influence in the more distant maternal relationships ($P = 0.03$). This may be a sampling bias, where distant relatives, whom the proband twins know less well, are more likely to be classified as MZ. Two other relationships that could be expected to show a DZ-influence are the offspring of the proband's sisters, and sibs of the mother.

Risk ratios for sisters calculated from these data (range 1.56–1.86) are substantially lower than the value from Bulmer [1970] of 2.6, from 4 studies that gave values of 2.2–3.0. The risk ratios from the offspring of proband twins was 2.7, which is substantially higher than the value of 1.8 of Bulmer [1970]. A more recent study of DZ probands in Belgium and the Netherlands gave a relative risk of 1.77 in sisters of proband mothers and 1.93 for the mothers of the probands. Although these figures show a wide range of risk ratios, each set of studies has its own problems with sampling bias or under-reporting of singleton births, and they all indicate a substantial increased risk in close female relatives of DZ twins.

The genetic influence on DZ twinning is clearly discernible in our study, even without accounting for maternal age and parity that strongly influence DZ twinning rates. The consistently elevated relative risks to sisters and mothers of women who have had spontaneous DZ twins found in this and other studies, together with segregation analysis results indicating a dominant mode of inheritance [Meulemans et al.,1993], suggest that DZ twinning may be a tractable (though far from ideal) trait for linkage studies through which genes with major influence on female fertility and infertility might be detected.

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